Original Research

Morphometry of Feline Adipose Tissue Derived Mesenchymal Stem Cells in Culture

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Abstract

Morphometric analysis of feline adipose derived mesenchymal stem cells (fAD-MSCs) was done at P1 and P2 (24 and 48 hours) of culture expanded using cover slip culture technique to record the morphometric details. In all the culture, spindle cells with long thin cytoplasmic processes were found to be more in number. The measure of length and width of cell and length and width of nucleus was done. There were no significant differences in both length and width of the cell and length and width of the nucleus between 24 and 48 hours of culture condition in P1 and P2.

Key words: Adipose Tissue and Culture, Feline, Mesenchymal Stem Cells, Morphometry

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Introduction

Stem cells were functionally defined as undifferentiated, primitive cells that had the capability to reproduce themselves for an indefinite period and also had the ability to generate multiple types of cells by receiving the proper signal from internal and external pathways (pluripotency or multipotency) (Sung Min *et al*., 2010). Unfortunately, unlike Embryonic stem cells (ESCs), most MSCs have a limited number of replications. The proliferation rate for Mesenchymal stem cells (MSCs) has been somewhat high compared to most cell types, but eventually the cells senesce. Only small subsets of MSCs had shown to undergo long term self-renewal in culture (Hao *et al.*, 1996). MSC had been isolated from tissues other than bone marrow,

as periosteum, trabeculae bone, adipose tissue, skeletal muscles, lung, peripheral blood, umbilical cord blood and placenta. All those cells were MSCs, but showed some differences related to proliferation and differentiation capacity (Kern *et al.,* 2006).

Adipose tissue composed of adipocytes that produced connective tissue matrix also contained nervous tissue, stroma vascular cells and immune cells (Frayn *et al.*, 2003). Adipose tissue, on the other hand represented a tissue source that was extremely abundant, readily accessible, resulted in minimal patient discomfort and yielded high enough cell numbers to sufficiently and efficiently expanded cell populations. Bone marrow had been investigated for a long time as the major source of MSCs. Adipose derived mesenchymal stem cells (AD-MSCs) were isolated from various species, including rodents (Ogawa *et al.,* 2004 and Yoshimura *et al.,* 2007) and swine (Qu *et al.,* 2007). These cells showed low levels of immunogenicity and had immunomodulatory properties (Poh *et al.,* 2007) and might be useful for allogeneic transplantation. According to Neupane *et al*. (2008) Quimby *et al*. (2011) and Spencer *et al.* (2012) there were considerable individual differences in mesenchymal stem cell numbers and growth reported in cats and dogs.

Materials and Methods

Collection of Feline Adipose Tissue

Omental adipose tissue samples were collected from female cats during ovariohysterectomy, in a sample collection bottle which contained phosphate buffer saline (PBS).

Isolation of Feline Adipose Derived Mesenchymal Stem Cells

By enzymatic digestion of the adipose tissue.

Seeding and Subculturing of Feline Adipose Derived Mesenchymal Stem Cells (fAD-MSCs)

The cells at a density of 6 x10^4 were plated in 6 well plates and added 2ml of culture media. In each well glass cover slip was added and incubated at 37°C and 5 per cent CO2 to cellular adherence. The cell culture was maintained for 24 and 48 hrs for passage 1(P1) and passage 2 (P2) (Fig. 1).

Staining Procedure of Feline Adipose Derived Mesenchymal Stem Cells

a) The culture media was removed from cell which was maintained 24 and 48 hrs.
b) Added two or three drops of Leishman's stain to cover slip.
c) Add two drops of distilled water.
d) Keep for 45 minutes, than wash cover slip and air dry.
e) Mounted the cover slip with DPX and observed under microscope

Then morphometry were measured - cell length and width and nucleus length and width (Maciel *et al.,* 2014). For morphometry one way ANOVA at 5 per cent level was used between various passage levels.

For analysing, values of P ≤0.05 were considered significant. All analysis was done by using SPSS-16. Statistical calculations (mean ± standard error) were recorded according to the standard statistical procedures recommended by Snedecor and Cochran (1994).

Result and Discussion

The fAD-MSCs expanded in cover slip culture technique at passage1 (P1) and passage 2 (P2) (24 and 48 hours of culture) were used for studying morphometric details. The fAD-MSCs were stained with Leishman's stain and observed the following cell types.

Fig 1 Photograph showing seeding of fAD-MSCs in six well plate

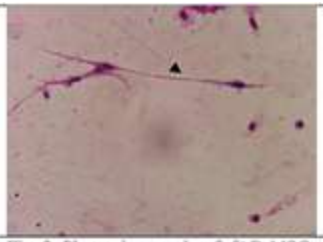

Fig 2 Photomicrograph of fAD-MSCs showing spindle shaped cells with long thin cytoplasmic processes (arrow) Leishman's Staining X 200

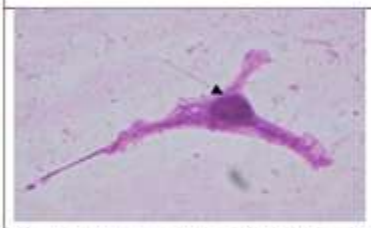

Fig 3 Photomicrograph of fAD-MSCs showing spindle shaped cells with Y-shaped cytoplasm (arrow) Leishman's Staining X 400

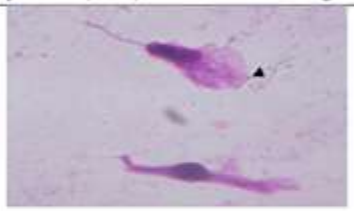

Fig 4 Photomicrograph of fAD-MSCs showing spindle shaped cells with cytoplasm at one end of the cell (arrow) Leishman's Staining X 400

Fig 5 Photomicrograph showing rounded widespread cells with abundant cytoplasm (arrow) Leishman's Staining X 400

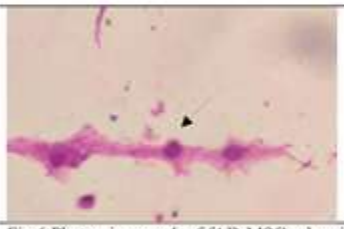

Fig 6 Photomicrograph of fAD-MSCs showing smaller spherical cell with a round centrally placed (arrow) Leishman's Staining X 200

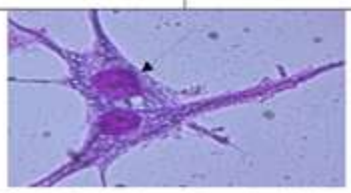

Fig 7 Photomicrograph of fAD-MSCs showing spindle cells with single and two nucleolus (arrow)

i. Spindle shaped cells with long, thin cytoplasmic processes at both ends of cells (Fig. 2).
ii. Spindle shaped cells with Y-shaped cytoplasm (Fig. 3).
iii. Spindle shaped cells with more cytoplasm at one end of the cell (Fig. 4).

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iv. Rounded widespread cells with abundant cytoplasm (Fig. 5).
v. Smaller spherical cell with rounded centrally placed nuclei (Fig. 6).
vi. Spindle shaped cells with single or double nucleolus (Fig. 7).

Of the above, spindle shaped cell with long, thin cytoplasmic processes were found to be numerous in all culture conditions. The length and width of fAD-MSCs, length and width of the nucleus were measured from cells at P1 and P2. The cell morphometry in P1 and P2 were shown in Table 1 and 2 respectively.

Table 1: Measurements (µm) of fAD-MSCs passage 1 (P1) - 24 and 48 hours of culture

Parameter	24 hours	48 hours
Cell length	98 ± 30.15	105.0 ± 33.20
Cell width	34.0 ± 15.25	35.0 ± 16.75
Nucleus length	15.10 ± 2.30	16.10 ± 2.50
Nucleus width	10.90 ± 2.10	11.71 ± 2.50

Table 2: Measurements (µm) of fAD-MSCs passage 2 (P2) - 24 and 48 hours of culture

Parameter	24 hours	48 hours
Cell length	109.0 ± 66.01	110.0 ± 70.07
Cell width	40.0 ± 19.01	45.18 ± 21.90
Nucleus length	19.00 ± 2.60	21.00 ± 3.00
Nucleus width	12.00 ± 2.40	15.21 ± 3.01

Groups do not different significantly with each other P > 0.05.

At P1 and P2, cell length and width increased without any statistical difference in 24 and 48 hours. The cell length was 98 ± 30.15µm and 105.0 ± 33.20 µm at 24 and 48 hours respectively in P1. In passage 2 (P2) cell length and width was 110.0 ± 66.01µm and 36.0 ± 17.01µm in 24 hours. Nucleus length and width was increased in P1, 15.10 ± 2.30 µm and 10.90 ± 2.10 µm in 24 hours without any statistical difference (Graph 1 and 2). There was no significant difference in both length and width of cell and also length and width of nucleus between 24 and 48 hours of culture condition in P1 and P2. This indicated that expansion of fAD-MSCs between passages occurred without change in cell morphology. Similar finding was observed by Sekiya *et al*. (2002), Docheva *et al*. (2008) in human MSCs and Maciel *et al*. (2014) in feline. Grzesiak *et al*. (2011) observed no statistical difference in morphometry of equines and canine AD-MSCs.

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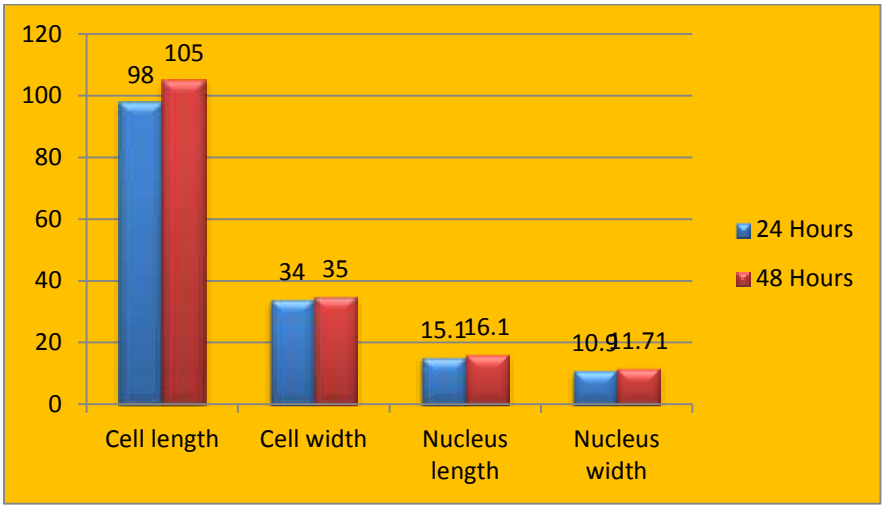
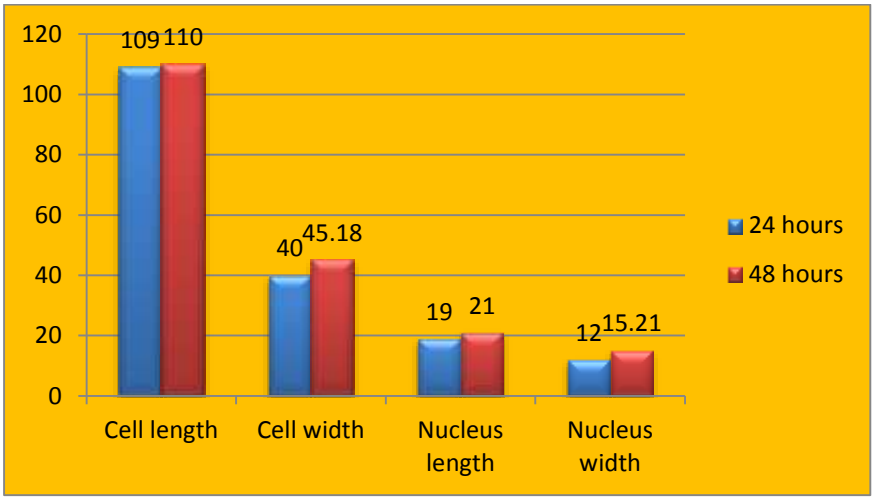


Graph 1: Graphical representation showing morphometry (µm) of feline adipose derived MSC at Passage 1 (P1) at 24 and 48 hours of culture



Graph 2: Graphical representation showing morphometry (µm) of feline adipose derived MSC at Passage 2 (P2) at 24 and 48 hours of culture

Conclusion

In the present study, the fAD-MSCs at P1 and P2 (24 and 48 hours) of culture using cover slip culture technique were used to record the morphometric details. The cell length and width and length and width of the nucleus were measured. There was no significant difference between the cell and nucleus. There is less information about morphology of fAD-MSCs, in present study we have observed spindle shaped were more predominant.

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